FIG. 1

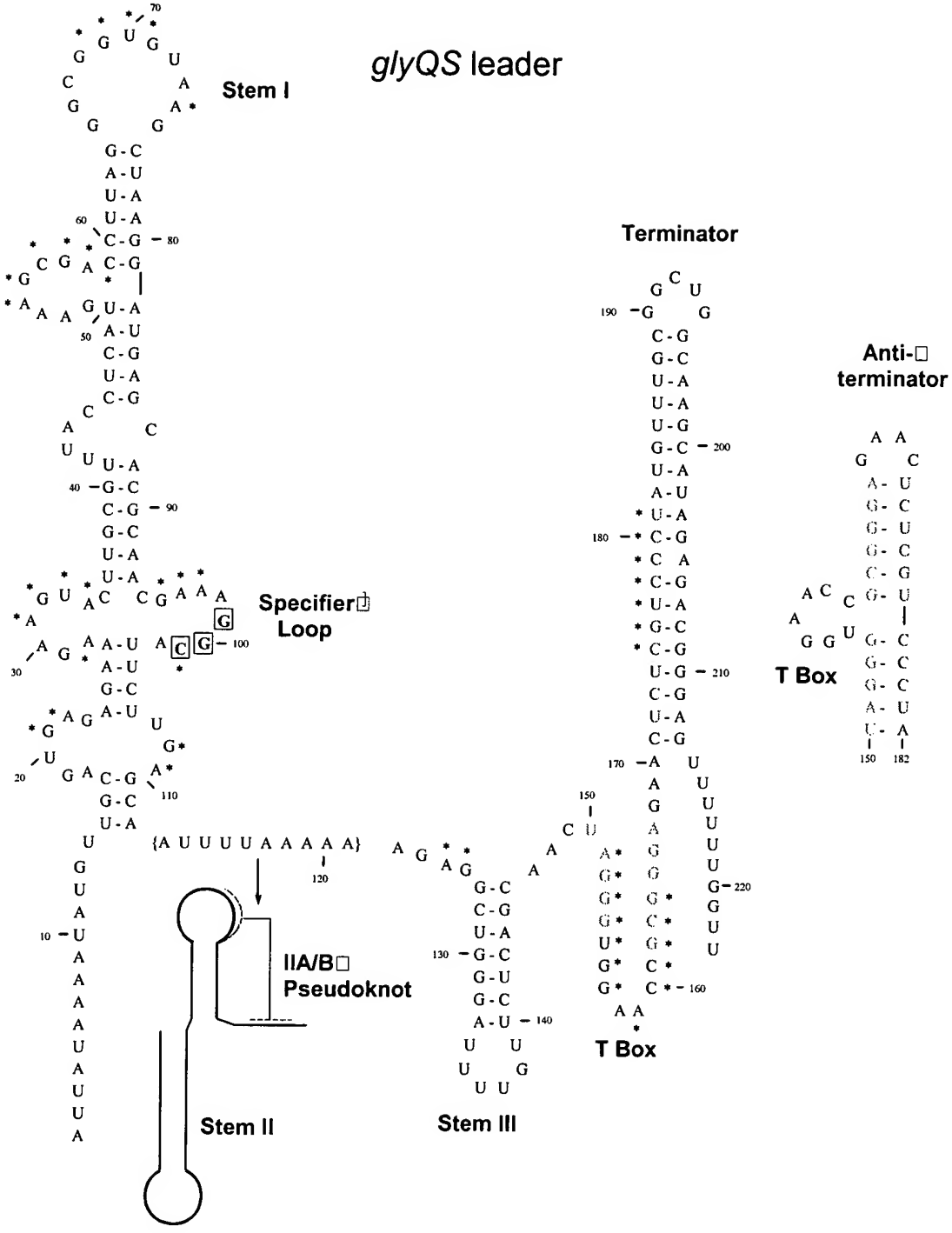
glyQS leader
Stem I
Terminator
Anti-□ terminator
Specifier□ Loop
T Box
IIA/B□ Pseudoknot
T Box
Stem II
Stem III

FIG. 2

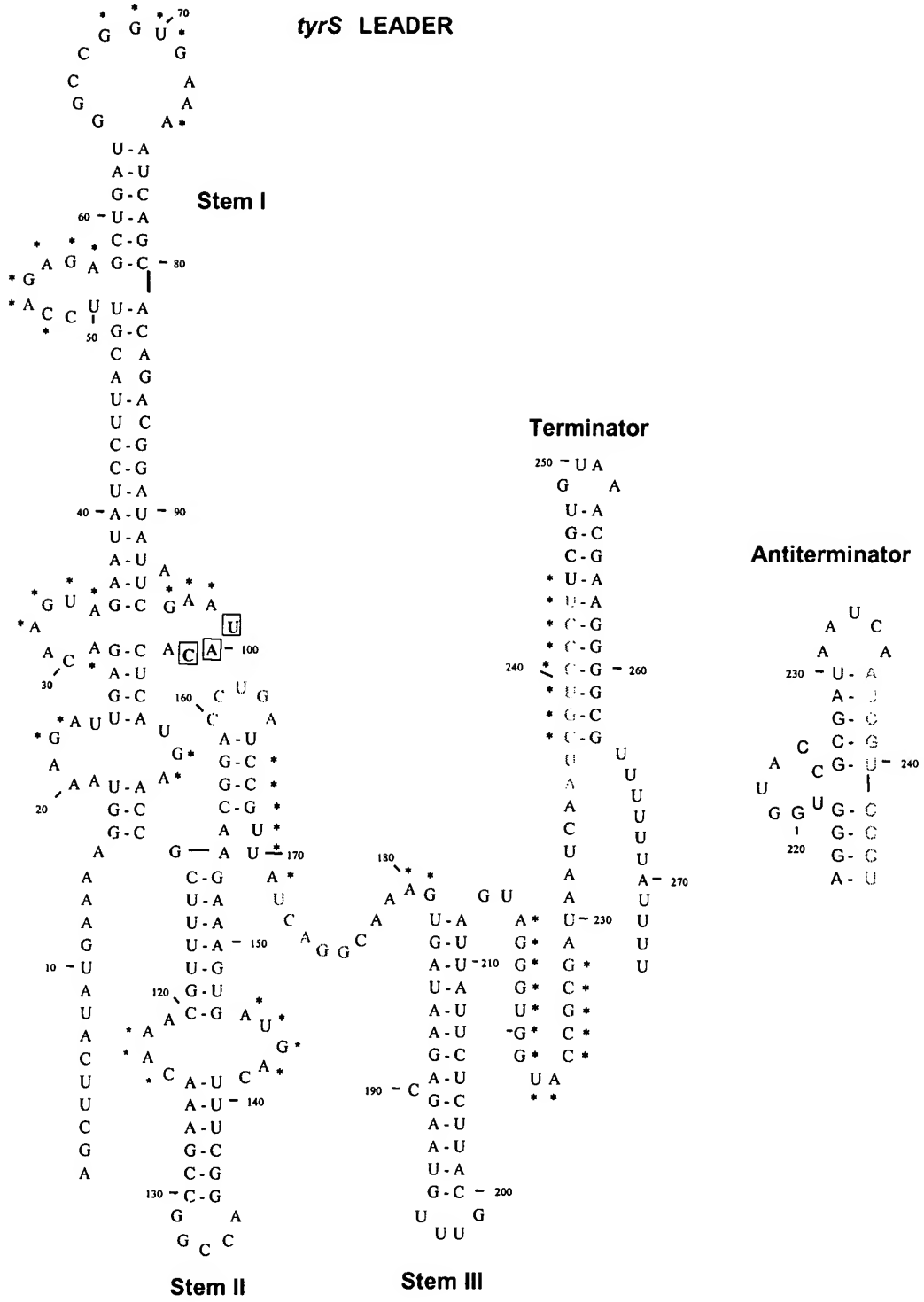
tyrS LEADER
Stem I
Terminator
Antiterminator
Stem II
Stem III

FIG. 3: Panel A

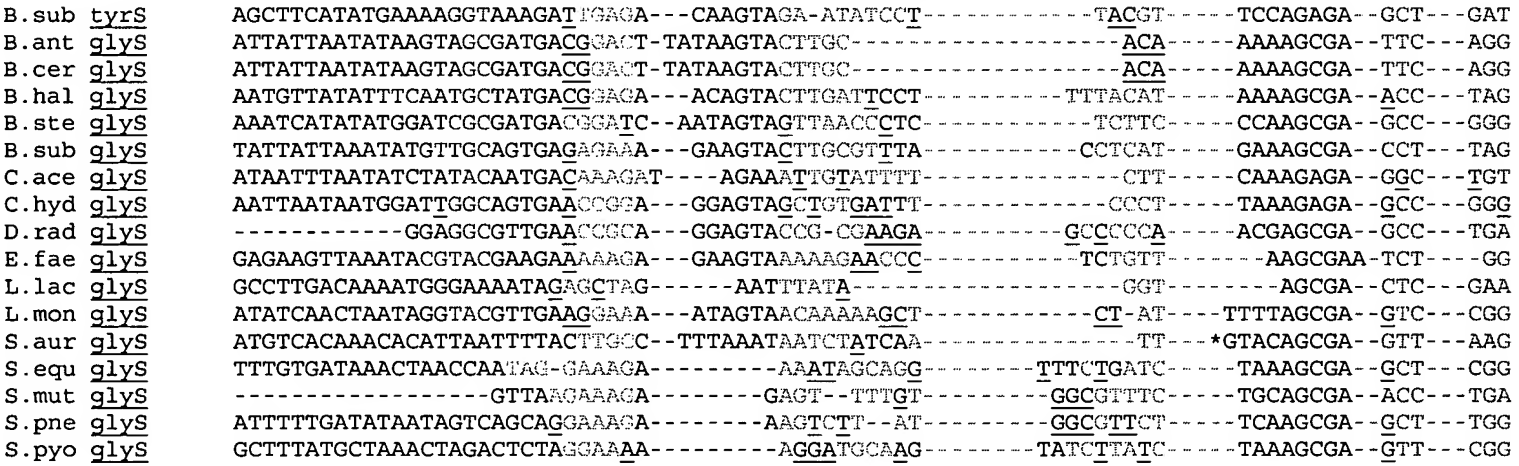

FIG. 3: Panel B

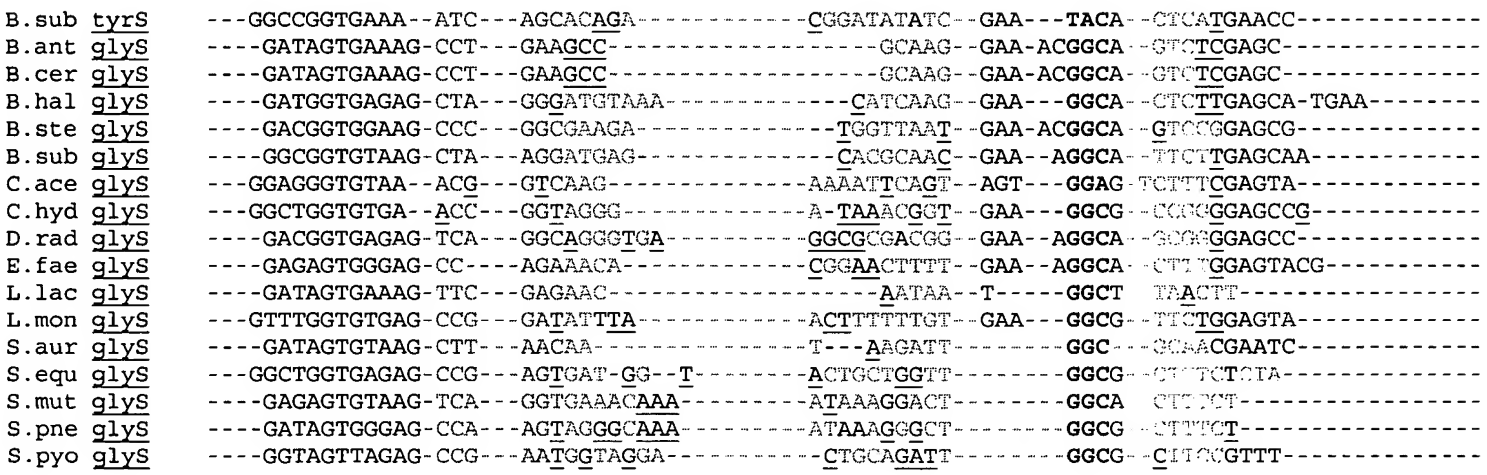

FIG. 3: Panel C

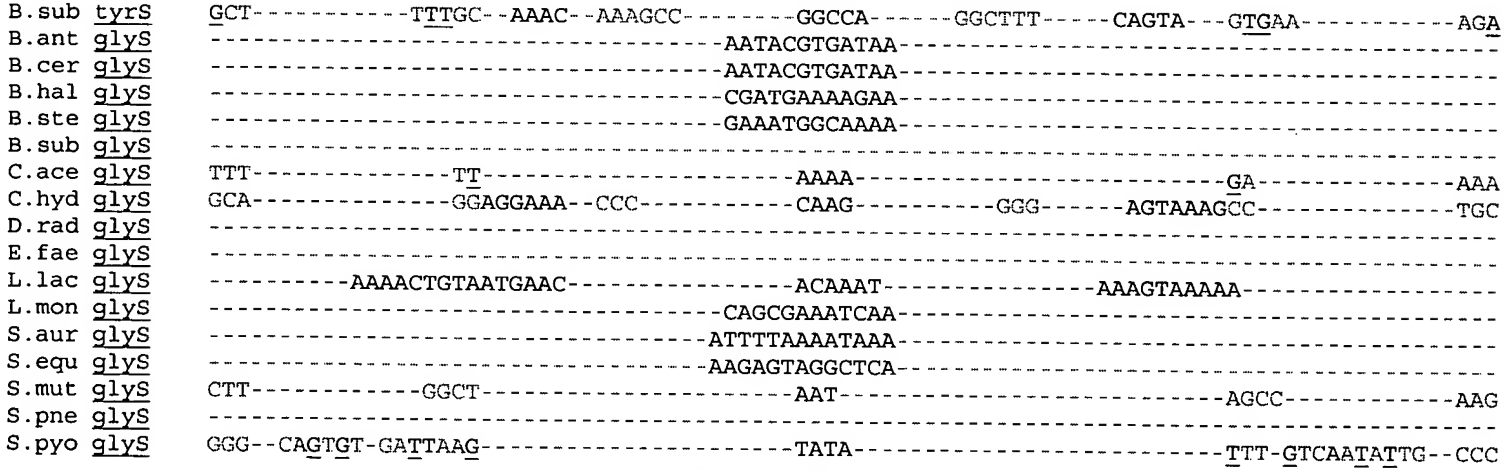

FIG. 3: Panel D

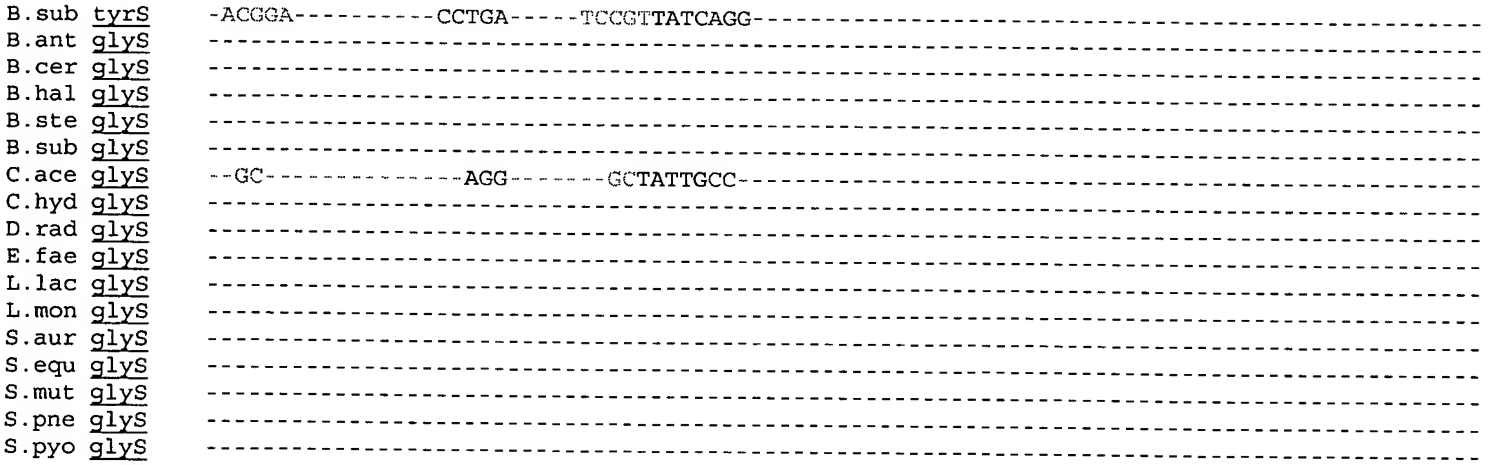

FIG. 3: Panel E

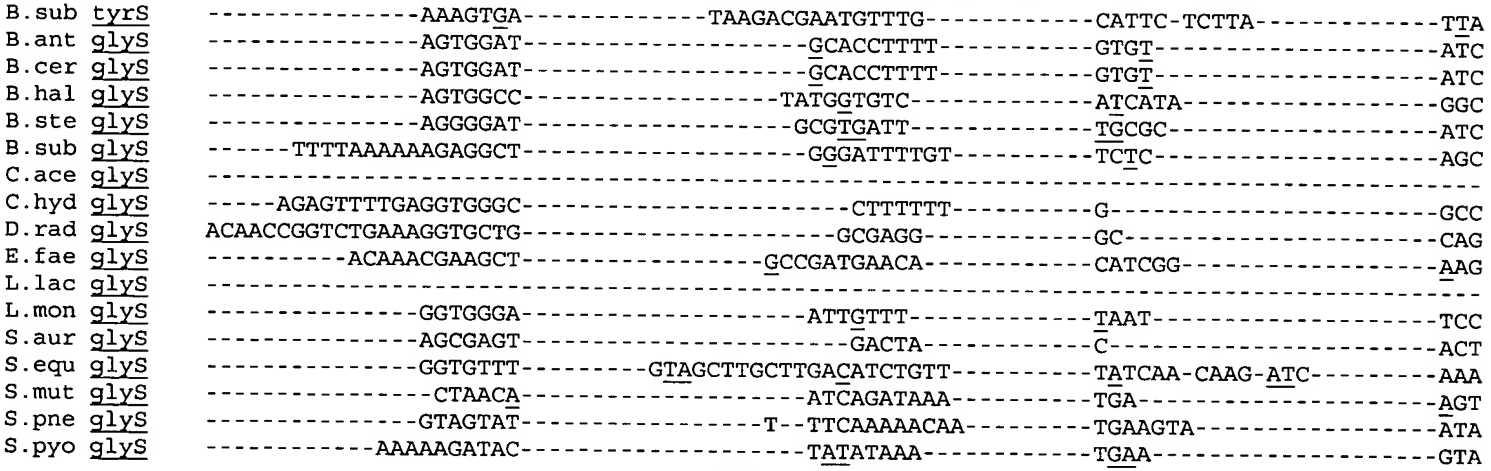

FIG. 3: Panel F

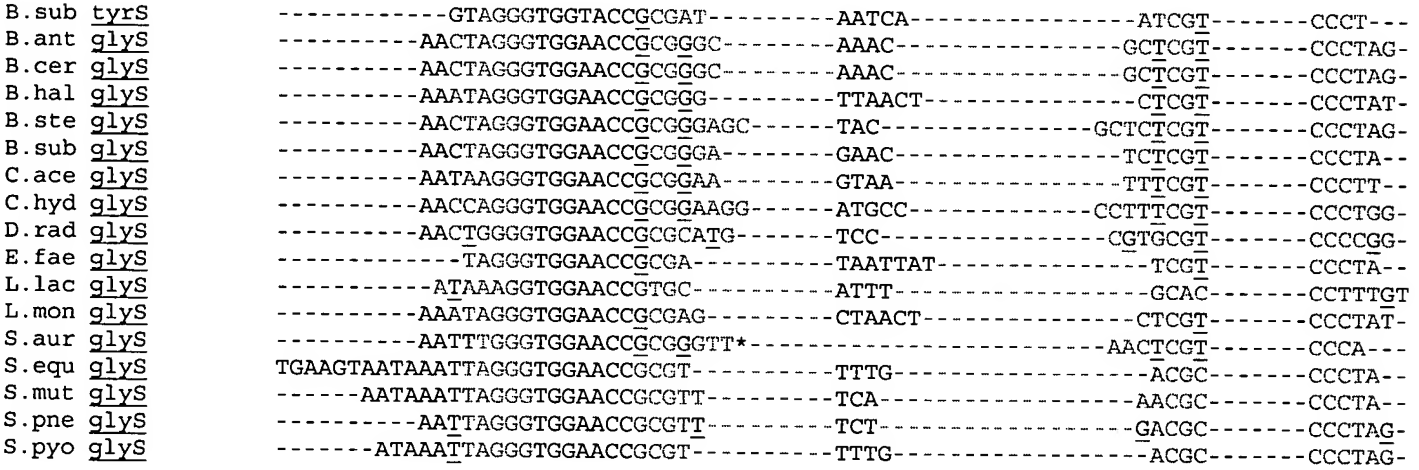

FIG. 4

ORGANISMS WITH LEADER SEQUENCES HAVING THE T BOX PATTERN	NCBI GENOME SEQUENCE FILE NAME
Bacillus anthracis	NC_003997
Bacillus cereus	NC_004722
Bacillus halodurans	NC_002570
Bacillus subtilis	NC_000964
Carboxydothermus hydrogenoformans	NC_002972
Clostridium acetobutylicum	NC_003030
Clostridium botulinum	NC_003223
Clostridium difficile	NC_002933
Clostridium perfringens	NC_003366
Clostridium tetani	NC_004557
Clostridium thermocellum	NZ_AABG00000000
Corynebacterium glutamicum	NC_003450
Corynebacterium diptheriae	NC_002935
Deinococcus radiodurans	NC_001263
Desulfitobacterium hafniense	NZ_AAAW00000000
Enterococcus faecalis	NC_004668
Enterococcus faecium	NZ_AAAK00000000
Geobacillus stearothermophilus	NC_002926
Geobacter sulfurreducens	NC_002939
Lactococcus lactis	NC_002662
Leuconostoc mesenteroides	NZ_AABH00000000
Listeria innocua	NC_003212
Listeria monocytogenes	NC_003210
Mycobacterium leprae	NC_002677
Mycobacterium tuberculosis	NC_000962
Oceanobacillus iheyensis	NC_004193
Oenococcus oeni	NZ_AAAAZ00000000
Ruminococcus albus	NC_003373
Spiroplasma kunkelii	NC_003999
Staphylococcus aureus	NC_002758
Staphylococcus epidermidis	NC_004461
Streptococcus equi	NC_002955
Streptococcus gordoniae	NC_002979
Streptococcus mutans	NC_004350
Streptococcus pneumoniae	NC_003098
Streptococcus pyogenes	NC_002737
Streptomyces coelicolor	NC_003888
Thermoanaerobacter tengcongensis	NC_003869
Thermobifida fusca	NZ_AAAQ00000000

FIG. 5

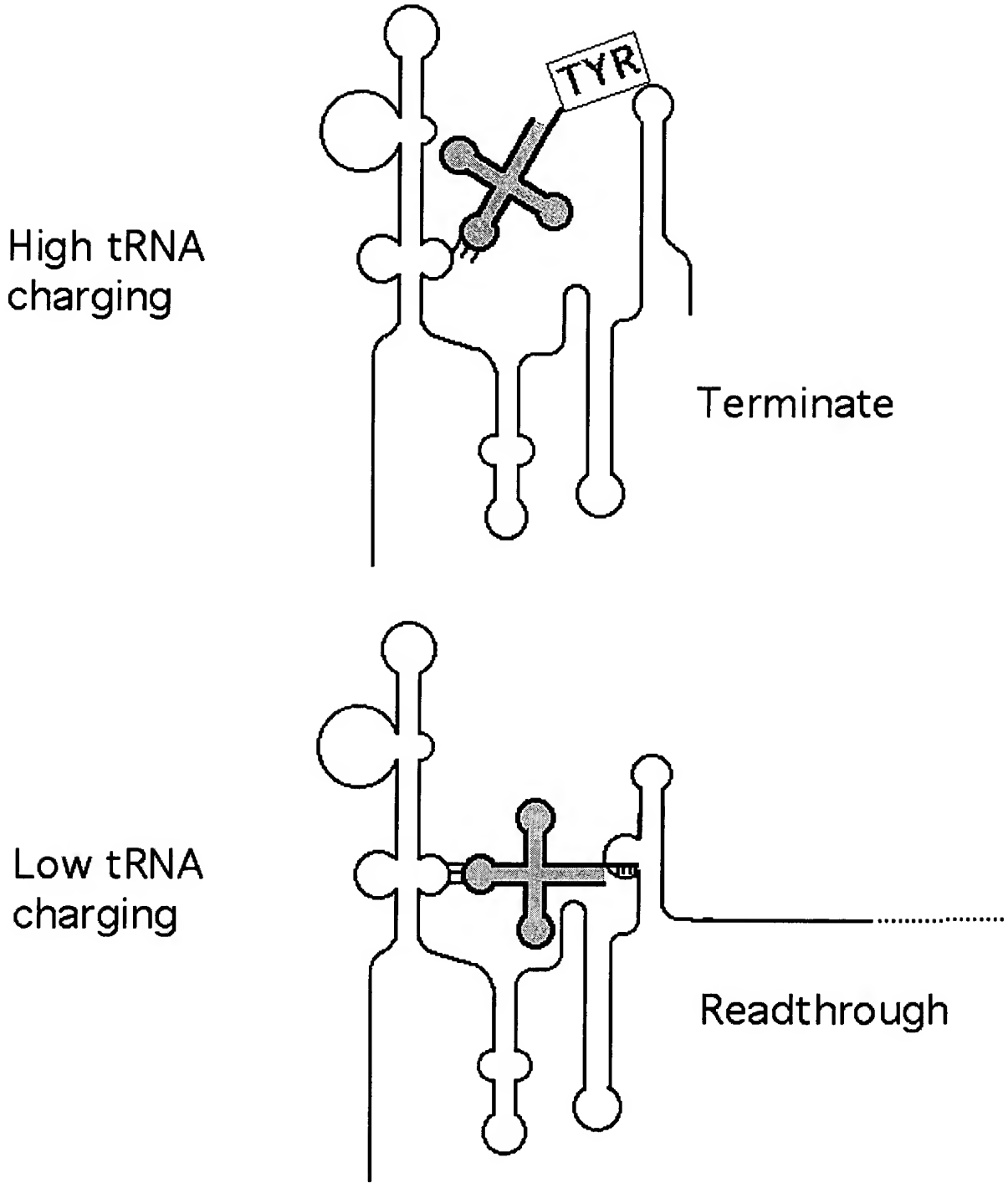
High tRNA charging
TYR
Terminate
Low tRNA charging
Readthrough

FIG. 6

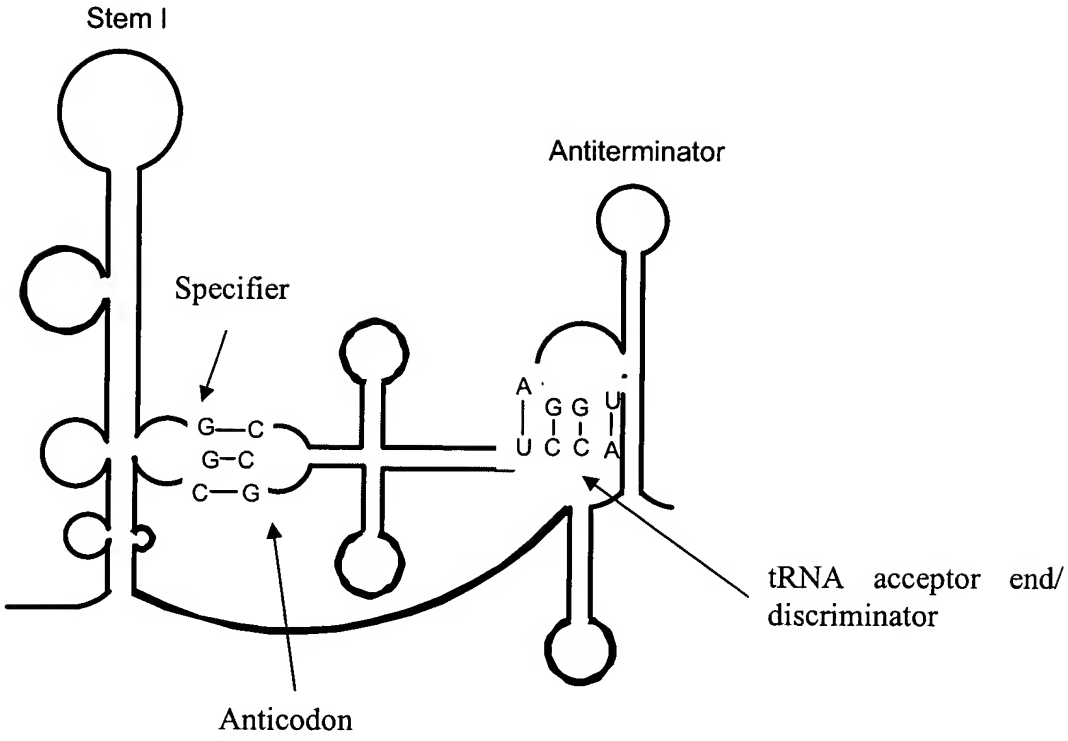
Stem I
Antiterminator
Specifier
G—C
G—C
C—G
A
G G U
U C C A
tRNA acceptor end/
discriminator
Anticodon

FIG. 7

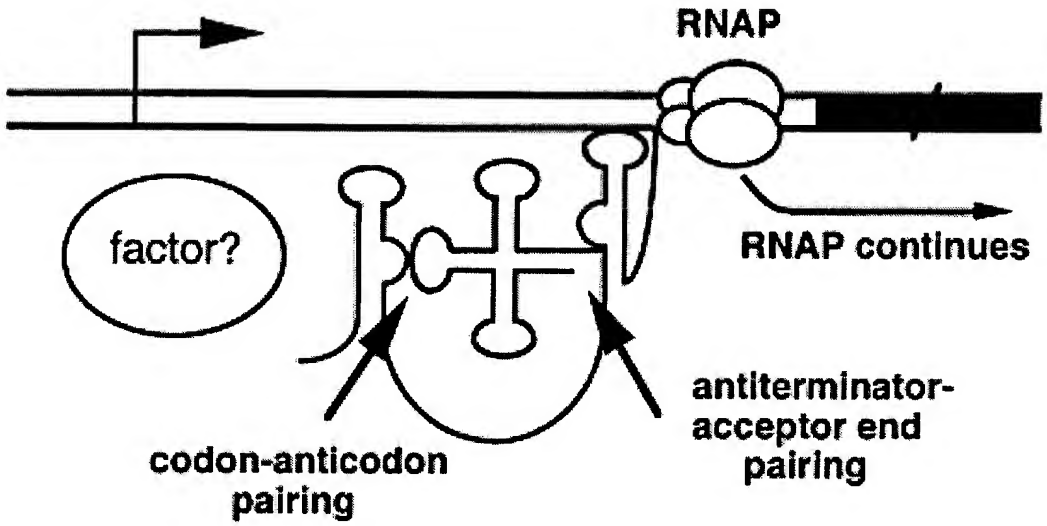
RNAP
factor?
RNAP continues
antiterminator-
acceptor end
pairing
codon-anticodon
pairing

FIG. 8

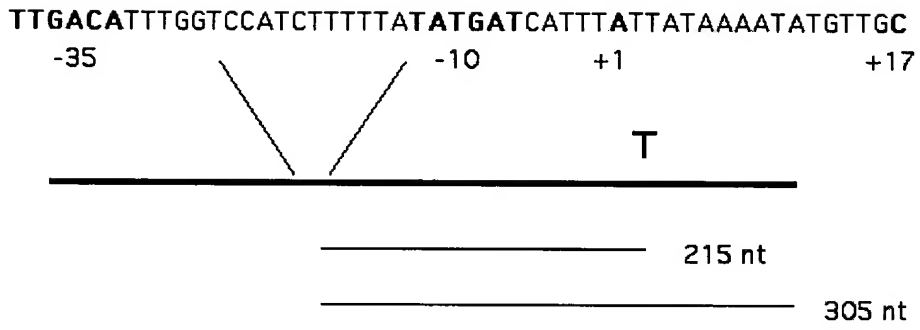
TTGACATTTGGTCCATCTTTTTATATGATCATTTATTATAAAATATGTTGC
-35
-10
+1
+17
T
215 nt
305 nt

FIG. 9

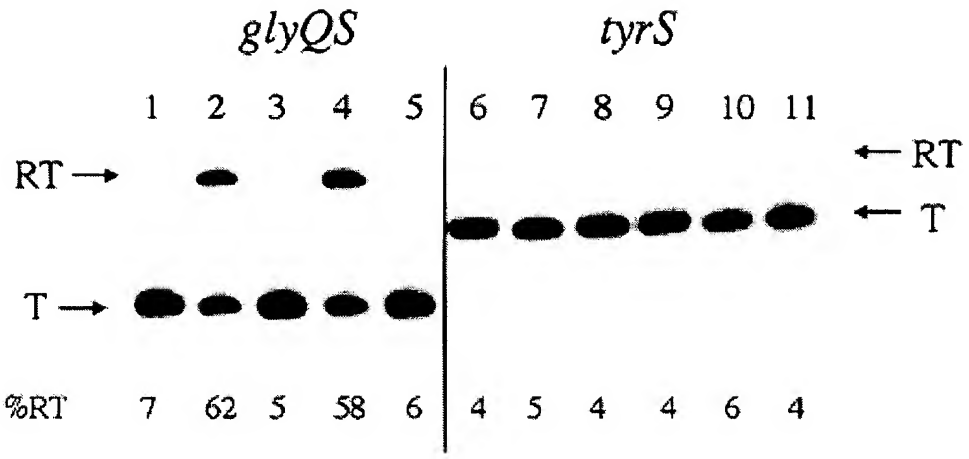

FIG. 10

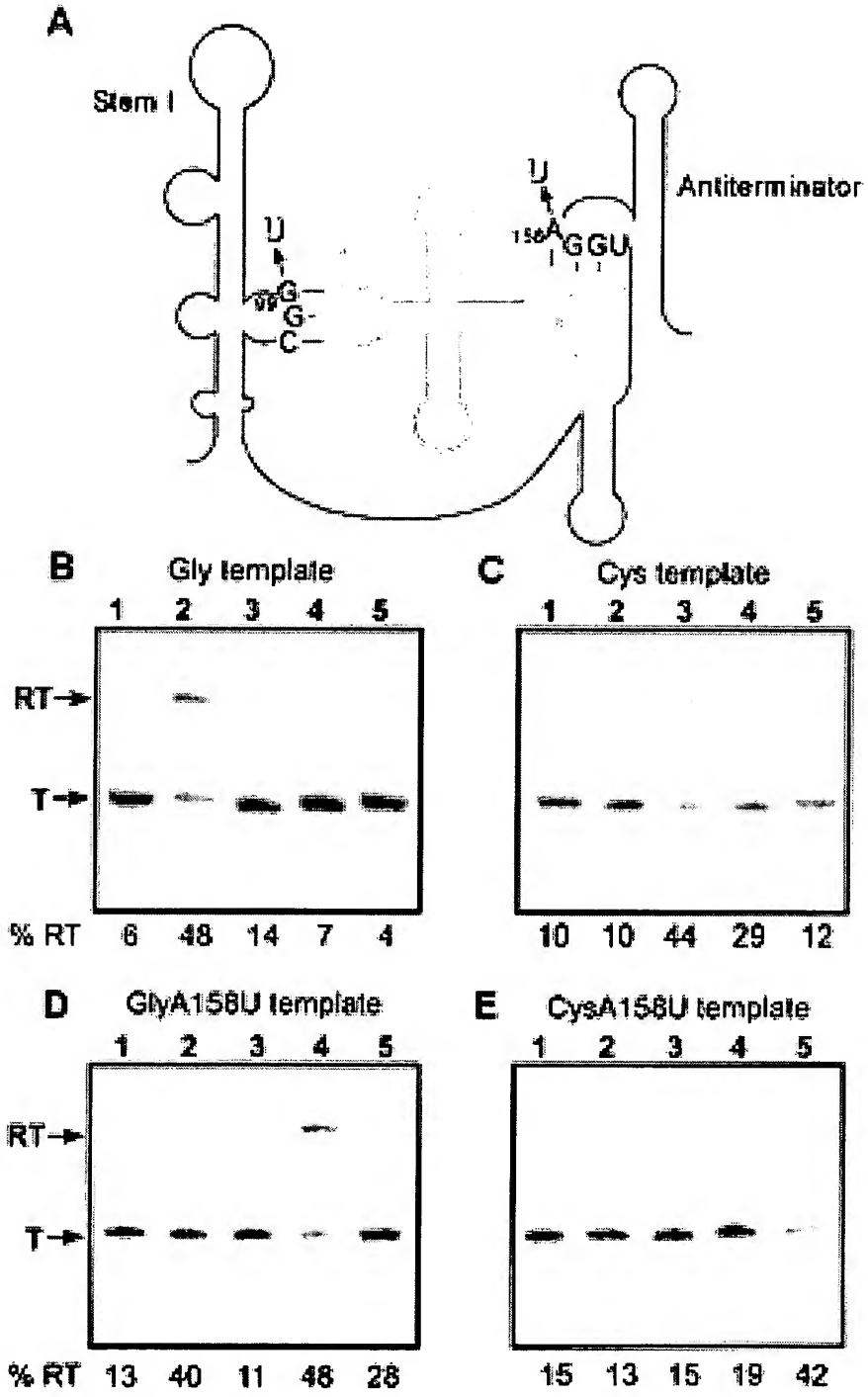
A
Stem I
Antiterminator
B
Gly template
C
Cys template
D
GlyA158U template
E
CysA158U template
RT
T
% RT
6 48 14 7 4
10 10 44 29 12
13 40 11 48 28
15 13 15 19 42

FIG. 11

Polynucleotide sequence for the *glyQS* gene from *Bacillus subtilis* corresponding to the *in vitro* transcription template: from *B. subtilis* 168.

ATTGATTTATATTACGAAGAATATTCGGGATTGTATTTAAAATCAAAGCGCTTTTTAGATCAAATGGAAAGCATGAA
ACATCTTATGGGTGAAAACAAAAGTTGACATTTGGTCCATCTTTTTATATGATCATTTATTATTAAATATGTTGCAG
TGAGAGAAAGAAGTACTTGCGTTTACCTCATGAAAGCGACCTTAGGGCGGTGTAAGCTAAGGATGAGCACGCAACGA
AAGGCATTCTTGAGCAATTTTAAAAAAGAGGCTGGGATTTTGTTCTCAGCAACTAGGGTGGAACCGCGGGAGAACTC
TCGTCCCTATGTTTGCGGCTGGCAAGCATAGAGACGGGAGTTTTTTGGTTGCTGCCGCAGTCAACTTATGAAAGAAA
AGTGGAGGTGCTTGAAATGAATATTCAAGACATGATTCTAACCTTGCAAAAGC

FIG. 12

Sequence of same region from BR151MA (T to A at +6 position relative to transcription start-site)

ATTGATTTATATTACGAAGAATATTCGGGATTGTATTTAAAATCAAAGCGCTTTTTAGATCAAATGGAAAGCATGAA
ACATCTTATGGGTGAAAACAAAAGTTGACATTTGGTCCATCTTTTTATATGATCATTTATTATAAAATATGTTGCAG
TGAGAGAAAGAAGTACTTGCGTTTACCTCATGAAAGCGACCTTAGGGCGGTGTAAGCTAAGGATGAGCACGCAACGA
AAGGCATTCTTGAGCAATTTTAAAAAAGAGGCTGGGATTTTGTTCTCAGCAACTAGGGTGGAACCGCGGGAGAACTC
TCGTCCCTATGTTTGCGGCTGGCAAGCATAGAGACGGGAGTTTTTTGGTTGCTGCCGCAGTCAACTTATGAAAGAAA
AGTGGAGGTGCTTGAAATGAATATTCAAGACATGATTCTAACCTTGCAAAAGC

FIG. 13

$tRNA^{Gly}$ DNA sequence: (from SubtiList, confirmed by sequencing of region in BR151MA)

GCGGAAGTAGTTCAGTGGTAGAACACCACCTTGCCAAGGTGGGGGTCGCGGGTTCGAATCCCGTCTTCCGCTCCA

FIG. 14

PCR primers used for preparing *glyQS* template:

GlyQUS1Xba: ATTGATCTAGATTACGAAGAATATTCGGGATTGTA (contains two changes from sequence shown in Fig. 12 to introduce an XbaI site (TCTAGA) at the 5' end of the fragment for generation of the *glyQS-lacZ* fusion construct)

GlyQDS2H3Pac: GGGTATTTAATTAAGCTTTTGCAAGGTTAGAATCA (introduces extra 14 nt downstream of *glyQS* sequence shown in Fig. 12 to provide a HindIIIsite (AAGCTT) for generation of the *glyQS-lacZ* fusion construct)

The PCR primers used to generate the *tyrS* template:

TyrUSBM: GGCTGGGGATCCGTCAACAATGGAGG
TyrN2b: CCGCGGAAGGATAAAGCTTCAAGTAAG

FIG. 15

Polynucleotide sequence for the *tyrS* template DNA (identical for 168 or BR151MA).

GGCTGGAGATCTGTCAACAATGGAGGATTAAAAGGCGGCGTTGACACAGGATTTTATTTATGTTAAAAATGATATAG
CTTCATATGAAAAGGTAAAGATTGAGACAAGTAGAATATCCTTACGTTCCAGAGAGCTGATGGCCGGTGAAAATCAG
CACAGACGGATATATCGAATACACTCATGAACCGCTTTTGCAAACAAAGCCGGCCAGGCTTTCAGTAGTGAAAGAAC
GGACCTGATCCGTTATCAGGCAAAGTGATAAGACGAATGTTTGCATTCTCTTATTAGTAGGGTGGTACCGCGATAAT
CAATCGTCCCTTCGTGTAAACGAAGGGGCGTTTTTTATTTTAATTAAAAAAGGAGCTTTATCTTATGACTAACTTAC
TTGAAGACTTATCCTTCCGCGG

FIG. 16

The oligos used to generate $tRNA^{Tyr}$:

```
5':  TyrUSBM:  GGCTGGGGATCCGTCAACAATGGAGG
3':  TyrN2b:  CCGCGGAAGGATAAAGCTTCAAGTAAG


5':  tRNATyrUST7:  TAATACGACTCACTATAGGAGGGGTAGCG (the tRNA sequence
starts with GGAGGGGTAGCG)
3':  tRNATyrDS:  TGGTGGAGGGGGGCAGATTCG
```

The oligos used to generate $tRNA^{Gly}$:

```
5':  GlytRNAUS:  TAATACGACTCACTATAGCGGAAGTAGTTCAGTGG
(the first part of this oligo is the T7 promoter; the tRNA starts
with GCGGAAGTAGTTCAGTGG)
3':  GlytRNADS:  TGGAGCGGAAGACGGGATTCGAAC
```

FIG. 17

$tRNA^{Tyr}$ DNA sequence.

GGAGGGGTAGCGAAGTGGCTAAACGCGGCGGACTGTAAATCCGCTCCCTCAGGGTTCGGCAGTTCGAATCTGCCCCC
CTCCACCA